equally important goal is to characterize protective immune responses (e.g. $CD4^+$ and $CD8^+$ T-cell responses, neutralizing antibody responses) as it has been found that low levels of pre-existing neutralizing antibodies to a subject's own infecting virus isolate do not necessarily protect from symptomatic DV infection. We aim to prospectively identify host-specific factors (e.g. pre-existing memory T and B cell responses to DV, HLA genetic polymorphisms, viral burden and replication in the host), virus-specific factors (e.g. DV serotype, serotype infection sequence), and environmental factors (e.g. mosquito population patterns, mosquito viral burden) for asymptomatic and symptomatic secondary DV infections, particularly severe infections (DHF/DSS). Multi-year investigations are planned to study the year-to-year variations in the incidence and prevalence of circulating serotypes.

The study employs school- and village-based components. Approximately 2000 children from 11 primary schools are actively followed for acute symptomatic DV infections. A 1-dilution screening neutralization assay is performed bracketing each dengue season to identify asymptomatic DV infections. Selected school-derived positive and negative dengue RT-PCR results from acute specimens serve to establish index cases for the initiation of cluster investigations within specific villages, the houses of which have been pre-mapped by GIS and pre-characterized as to number of children and their ages. Upon initiation of a cluster investigation, 10-25 child contacts (6mo-15 yrs of age) are identified within a 100-meter radius of the index case and are evaluated by questionnaire serially for 15 days for DV infection with blood samples taken on days 0 and 15 regardless of illness. Mosquitoes are collected within the 100-meter radius and are tested utilizing dengue RT-PCR. Mosquito spraying is further conducted to halt local DV transmission.

The methodology of this multi-collaborative study will be described to include quality assurance (QA) systems employed to have this study serve as a bridging study to the future testing of dengue vaccines in phase 2 and phase 3 clinical trials.

1st Regional Meeting of Pediatric Dengue Vaccine Initiative (PDVI). Bangkok, Thailand. 18-20 October 2004.

THE MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS SEROTYPE 2 AND 3 CIRCULATING IN THAILAND FROM 1974 TO 2001

Zhang C, Mammen MP Jr, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P and Holmes EC

Dengue represents a major public health problem in Thailand, with all four viral serotypes co-circulating. This provides an optimal setting to investigate the molecular epidemiological history and evolutionary trends of these co-circulating dengue virus (DENV) serotypes in a highly endemic country; to characterize intra-serotypic variations of DENV in a locality; to determine the evolutionary forces shaping viral genetic diversity; to determine whether the changing prevalence of DENV could be attributed to instances of adaptive evolution in the viral genome. Furthermore, it permits the investigations of whether molecular determinants of the envelope (E) gene of DENV correlate with disease severity. To this end, we undertook a large-scale molecular epidemiological analysis of 173 E gene sequences of DENV representing

each of the 2 serotypes (105 DENV-2 and 68 DENV-3) isolated from children admitted from 1974 to 2001 in Thailand (Bangkok and Kamphaeng Phet) with varying degrees of dengue severity [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)]. Our results indicated that there was no obvious molecular correlate between disease severity and the phylogenetic position of their associated E genets. These analyses revealed extensive genetic diversity within a single geographic locality at a single time. The phylogenetic trees of DENV showed a strong temporal structure, ladder-like structure for both serotypes; viral strains isolated at the earliest time-points tended to fall near the root of the trees. These temporal orderings are caused by the continual birth and death of viral lineages; new lineages are regularly produced by mutation, but most go extinct relatively rapidly and few progress to circulate in subsequent years. Consequently, the rapid turnover of DENV lineages observed is, at most, the consequence of high, rates of deleterious mutations in the viral genome coupled with seasonal fluctuations in the size of the vector population.

Abstract of the Joint International Tropical Medicine Meeting (JITMM). Bangkok, Thailand. 29 November-1 December 2004:164.

MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS SEROTYPE 3 AND 4 IN BANGKOK, THAILAND

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Dengue represents a major public health problem in Thailand, with all four viral serotypes co-circulating. To determine the evolutionary forces shaping the genetic diversity of dengue virus serotype 3 (DENV-3) and serotype 4 (DENV-4), and in particular to determine whether the changing prevalence of DENV-3 and DENV-4 could be attributed to instances of adaptive evolution in the viral genome, we undertook a large-scale molecular epidemiological analysis of DENV-3 and -4 (60 DENV-3 and 53 DENV-4 isolates from children in Bangkok, Thailand, admitted with varying degrees of dengue severity [dengue fever/dengue hemorrhagic fever/ dengue shock syndrome (DF/DHF/DSS)] from 1974 to 2002) using both E gene sequences of sixty DENV-3 and fifty three DENV-4, and six complete viral genomes of DENV-4. These analyses revealed extensive genetic diversity within a single locality at a single time, including the discovery of a new and divergent genotype of DENV-4. However, despite this abundant genetic variation, there was no evidence of adaptive evolution in any gene, codon, or lineage of DENV-4. Consequently, the rapid turnover of DENV-3 and -4 lineages observed is, at most, the consequence of high rates of deleterious mutations in the viral genome coupled with seasonal fluctuations in the size of the vector population. (ACMCIP abstract)

53rd Annual Meeting of the American Society Tropical Medicine and Hygiene (ASTMH). Miami, Florida, USA. 7-11 November 2004.

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